

Heat shock protein 90 β inhibits apoptosis of intestinal epithelial cells induced by hypoxia through stabilizing phosphorylated Akt

Shuai Zhang, Yong Sun, Zhiqiang Yuan, Ying Li, Xiaolu Li, Zhenyu Gong & Yizhi Peng*

Institute of Burn Research, Southwest Hospital, State Key Laboratory of Trauma, Burns and Combined Injury, Third Military Medical University, Chongqing 400038, China

Intestinal epithelial cell (IEC) apoptosis induced by hypoxia compromise intestinal epithelium barrier function. Both Akt and Hsp90 have cytoprotective function. However, the specific role of Akt and Hsp90 β in IEC apoptosis induced by hypoxia has not been explored. We confirmed that hypoxia-induced apoptosis was reduced by Hsp90 β overexpression but enhanced by decreasing Hsp90 β expression. Hsp90 β overexpression enhanced BAD phosphorylation and thus reduced mitochondrial release of cytochrome C. Reducing Hsp90 β expression had opposite effects. The protective effect of Hsp90 β against apoptosis was negated by LY294002, an Akt inhibitor. Further study showed that Akt phosphorylation was enhanced by Hsp90 β , which was not due to the activation of upstream PI3K and PDK1 but because of stabilization of pAkt via direct interaction between Hsp90 β and pAkt. These results demonstrate that Hsp90 β may play a significant role in protecting IECs from hypoxia-induced apoptosis via stabilizing pAkt to phosphorylate BAD and reduce cytochrome C release. [BMB Reports 2013; 46(1): 47-52]

INTRODUCTION

The gastrointestinal tract performs many vital functions, including the processing and absorption of ingested nutrients, waste removal, fluid homeostasis, and the development of oral tolerance to nonpathogenic luminal antigens (1). Lining the entire gastrointestinal tract, intestinal epithelial cells (IECs) form a dynamic barrier against bacterial activation of the mucosal immune system via highly regulated cellular turnover and tight junction protein complexes (2). A balance between cellular proliferation and

apoptosis is necessary to maintain this critical barrier (3). Excessive IEC apoptosis compromises mucosal barrier function due to "apoptotic leak" (2). Intestinal epithelial barrier dysfunction contributes to the development of sepsis and multiple organ failure (4).

Hypoxia is associated with a number of ischemic conditions, including trauma, or severe burns (5). Due to high blood flow under normal conditions, the intestine is particularly susceptible to ischemia and resultant tissue hypoxia (6). It has been shown that ischemia/reperfusion triggers apoptosis in rat intestinal epithelial cells (7).

Hsp90, as a molecular chaperone, including two major isoforms, Hsp90 α and Hsp90 β , is one of the most abundant Hsps in eukaryotic cells (8). Hsp90 plays an essential role in the folding and activation of a range of client proteins involved in cell survival and certain signal transduction (9). Hsp90 can prevent cell apoptosis induced by cellular stresses (10) and Hsp90 inhibitors could induce apoptosis in various types of cells (11). However, the role of Hsp90 in IEC apoptosis under hypoxia remains undefined. Although the functional difference between Hsp90 α and Hsp90 β has not been well established, it is well known that Hsp90 α provides protection for many types of cells including IECs under stress (12). In contrast, if Hsp90 β exerts a cytoprotective effect against intestinal epithelial injury remains undefined.

The PI3K/Akt signaling pathway is a prototypic survival pathway (13). Akt phosphorylates a number of proapoptotic proteins including BAD and Forkhead transcription factors to suppress their proapoptotic activities (14). Akt seems to be an Hsp90-dependent kinase because its active form (pAkt) is stabilized by forming an intracellular complex with Hsp90 and Cdc37 (15). Inhibition of Hsp90-Akt interaction reduces pAkt and suppresses Akt kinase activity (16). However, in some cell types, activation of Akt or inhibition of PI3K has no effect on their survival under stress (17).

Although Hsp90 and Akt regulate cell apoptosis during hypoxia in some cell types, little is known about their roles in IEC survival under hypoxic condition and the underlying molecular mechanism(s), which are the topics of this study. Here, we show that hypoxia induces apoptosis of IECs, which can be suppressed

*Corresponding author. Tel: +86-23-68754175; Fax: +86-23-654 60398; E-mail: yizhipen@sina.com
<http://dx.doi.org/10.5483/BMBRep.2013.46.1.037>

Received 20 February 2012, Revised 3 May 2012,
Accepted 12 June 2012

Keywords: Apoptosis, Caco2 cells, Hypoxia, Intestinal epithelial cell, PI3K/Akt signaling pathway

by Hsp90 β overexpression but exacerbated by Hsp90 β knockdown. This is due to the stabilization of pAkt by Hsp90 β during hypoxia. These results may provide an insight into the pathogenic mechanisms of intestinal epithelial barrier dysfunction during intestinal hypoxia.

RESULTS

Effect of Hsp90 β on hypoxia-induced apoptosis of the Caco2 cells

To determine if hypoxia could induce apoptosis of the Caco2 cells, these cells were harvested at 0, 2, 3, 6, 12, 24 and 48 hours post hypoxia (1% O₂) and the percentage of apoptotic cells (apoptotic rate) was measured by the Annexin V-APC/PI Assay. Low percentages of Annexin V-positive cells were observed in the Caco2 cells under normoxia, whereas the exposure to hypoxia increased apoptotic rates as early as 3 h, with the apoptotic rate peaking at 24 h (Fig. 1A).

Caspase activation is a critical step in cell apoptosis. Thus caspase 3 activity was measured at 6, 12 and 24 hours after hypoxia. Compared with the control group, caspase 3 activity was increased significantly in the hypoxia group at times ≥ 6 h, with levels peaking after 12 h (Fig. 1B).

To investigate the effects of Hsp90 β on hypoxia-induced apoptosis of Caco2, the expression of Hsp90 β was either increased by AdHsp90 β infection by 3 folds or decreased by Hsp90 β shRNA

plasmid transduction by 2.5 folds (Fig. 1C, D). Compared with the hypoxia control group, the Caco2 cell overexpressing Hsp90 β had a significantly lower percentage of apoptotic rates measured by Annexin V-APC/PI staining and TUNEL staining. In accordance, the percentages of Annexin V-positive and TUNEL⁺ cells were increased in cells with Hsp90 β knockdown compared to the hypoxia control group (Fig. 1E, F). These data suggest that Hsp90 β exerts a protective role in Caco2 cell apoptosis induced by hypoxia.

Hsp90 β inhibits the activation of mitochondria-dependent apoptotic pathway

Hypoxia induced cell apoptosis is believed to be mediated by the mitochondria-mediated intrinsic apoptotic pathway. This process involves cytochrome C release from the mitochondria through the mitochondrial permeation transition pore (mPTP) comprising of BAD and other components. Phosphorylation of BAD by Akt reduces its ability to form mPTP (18). Caspase 3 activation is a common step of the apoptotic signaling pathway and caspase 3 mediated PARP cleavage precipitates cell apoptosis (19). In the Caco2 cells, hypoxia slightly increased the level of pBAD, indicating that the cell is trying to counteract the detrimental effects of hypoxia. Hsp90 β overexpression further increased pBAD abundance. In contrast, Hsp90 β shRNA decreased pBAD abundance. Accordingly, cytochrome C release from the mitochondria into the cytosol was decreased by

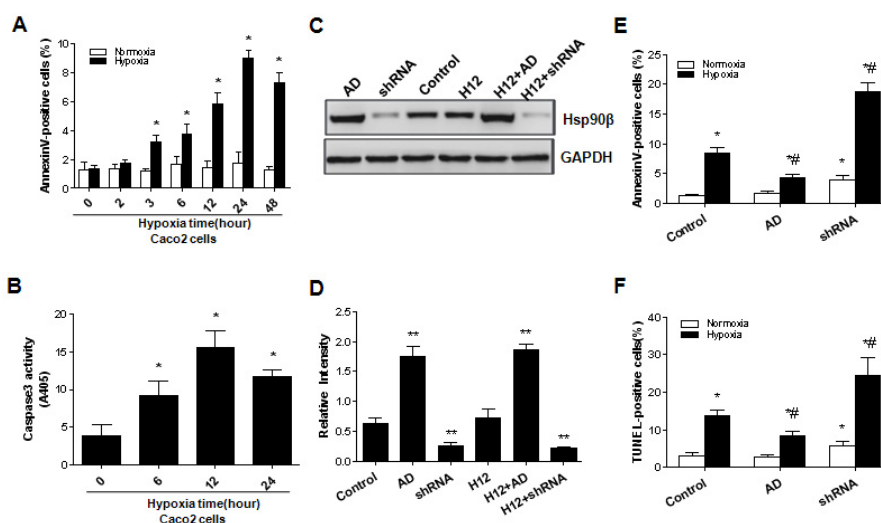


Fig. 1. Hsp90 β protects Caco2 cell apoptosis induced by hypoxia. The Caco2 cells were infected with Hsp90 β adenoviruses (AD) or transduced with shRNA plasmids against Hsp90 β . Then cells were subjected to hypoxia for 12 hours (H12). (A) The percentage of the Caco2 cells stained positive for Annexin V-APC/PI at different time points after hypoxia. Hypoxia induced the appearance of Annexin V, an early indicator of cell apoptosis, on the outer membrane of Caco2 cells in a time-dependent manner. * $P < 0.05$ vs. normoxia at the same time, two way ANOVA. (B) Caspase 3 activity at different times after hypoxia showing increased caspase 3 activity after hypoxia. * $P < 0.05$ vs. at time 0. (C, D) The cells were subjected to hypoxia 12 hrs. Hsp90 β levels were determined using Western blot. The intensity of each band was quantified by densitometry and GAPDH was used as the internal control. ** $P < 0.01$ vs. controls. (E) The percentages of cells positive for Annexin V-APC/PI staining in AD and shRNA groups of cells were shown. * $P < 0.05$ vs. control at the same treatment; # $P < 0.05$ vs. control cells with hypoxia. (F) The percentages of TUNEL⁺ cells in Caco2 cells with AD or shRNA under normoxic or hypoxic condition. * $P < 0.05$ vs. control at the same treatment; # $P < 0.05$ vs. control cells with hypoxia; $n = 3$.

Hsp90 β overexpression but reduced by Hsp90 β knockdown (Fig. 2A, B). Overexpression of Hsp90 β had no effect on the amount of cleaved caspase 3 and cleaved PARP in the Caco2 cells under normoxic condition. Hypoxia significantly increased the abundance of cleaved caspase 3 and PARP. When Hsp90 expression was reduced by shRNA, the amount of cleaved caspase 3 and PARP significantly increased 2-fold and 3-fold, respectively. Overexpressing Hsp90 β suppressed the cleavage of caspase 3 and PARP (Fig. 2C, D). Our results suggest that Hsp90 β inhibits the hypoxia-induced mitochondria-dependent apoptotic signaling pathway in the Caco2 cells.

The role of Akt in Caco2 cell apoptosis induced by hypoxia

Multiple protein kinases, including Akt, are involved in cell apoptosis during hypoxia (20). To determine which kinase is involved in Caco2 cell apoptosis during hypoxia, we treated the Caco2 cells under normoxic or hypoxic condition with a PKA inhibitor, an Akt inhibitor, a PKC inhibitor and a MAPK inhibitor. Among these inhibitors, only the Akt inhibitor promoted Caco2 cell apoptosis under hypoxia, suggesting that Akt plays an important role in regulating the hypoxia-induced apoptosis of Caco2 cells (Fig. 3A). In Hsp90 β overexpressing Caco2 cells, the Akt inhibitor completely abolished the protective effect of Hsp90 β on the Caco2 cells (Fig. 3B). This indicates that Akt is the main signal molecule involved in protecting IECs from apoptosis during hypoxia as in some cell types (21). We further studied if Akt activation is promoted by Hsp90 β . In the Caco2 cells, the total expression of Akt was not altered either by hypoxia or

by Hsp90 β expression. pAkt remained at a low level under normoxia and hypoxia led to an increase in pAkt. Overexpressing Hsp90 β in Caco2 cells under hypoxia further increased the level of pAkt but Hsp90 β knockdown decreased pAkt significantly (Fig. 3C, D).

Hsp90 β enhances pAkt not by activating upstream PI3K and PDK1 but by stabilizing pAkt in hypoxic Caco2 cells

To explore how Hsp90 β increases pAkt in Caco2 cells, we first examined the expression and activation of PI3K and PDK1 and their ability to activate Akt. Hsp90 β did not change the expression levels of the total PI3K and PDK1, nor did it change the phosphorylation of PI3K (p-PI3K) and PDK1 (p-PDK1) (Fig. 4A, B). These results suggest that Hsp90 β enhances pAkt not through upstream molecules, PI3K and PDK1.

It has been suggested that Hsp90 is able to stabilize and thus increases pAkt (22). In the Caco2 cells, Hsp90 β was co-immunoprecipitated with pAkt and conversely, pAkt was co-immunoprecipitated with Hsp90 β under normoxic and hypoxic conditions. Hypoxia tended to increase the amount of Hsp90 β -pAkt complex. Hsp90 β -overexpression increased the amount of Hsp90 β -pAkt complex but Hsp90 β shRNA group reduced the amount of this complex in the hypoxic group (Fig. 4C-F).

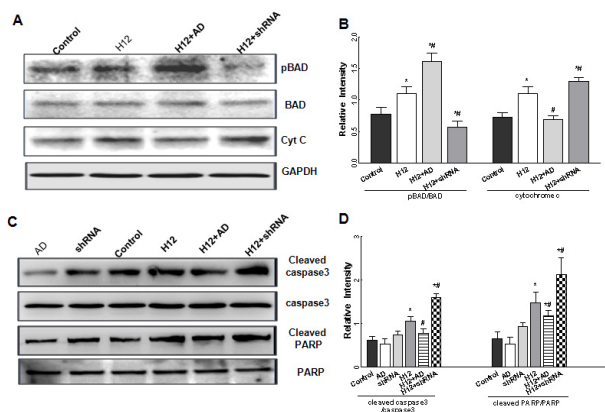


Fig. 2. Hsp90 β regulates the mitochondria-dependent apoptotic pathway. (A, B) Western blots of cytosolic cytochrome C (cyt C), pBAD and BAD. (C, D) Western blot analyses detecting the expressions of cleaved caspase 3 and cleaved PARP in the Caco2 cells under normoxic and hypoxic conditions. Graph represents the mean \pm SEM (n = 3) of the relative integrated signals. AD, over-expression of Hsp90 β using adenovirus; shRNA, Hsp90 β knockdown using shRNA plasmids; H12, hypoxia (1% O₂) for 12 hrs. *P < 0.05, versus normoxic controls; #P < 0.05, versus hypoxia control group.

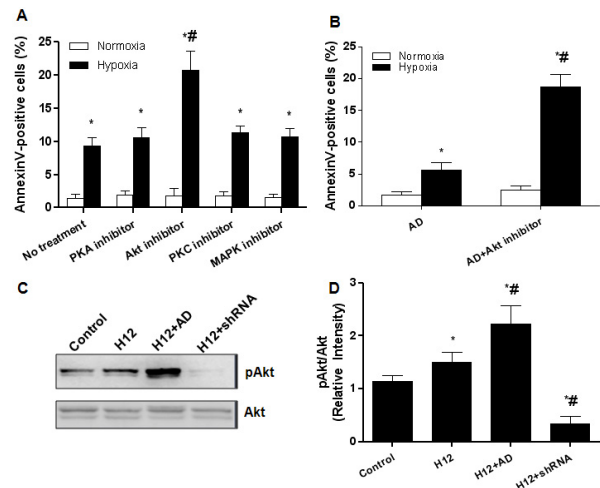


Fig. 3. Akt protects hypoxic Caco2 cells from apoptosis and Hsp90 β promotes hypoxia-induced Akt phosphorylation. (A) Effects of kinase inhibitors on hypoxia induced Caco2 cell apoptosis evaluated by Annexin V-APC/PI staining showing only Akt inhibition had an effect. (B) Akt inhibition abolished protective effects of overexpressed Hsp90 β in Caco2 cells under hypoxia. (C, D) Examples and the quantitation of Western blots of pAkt and Akt in Caco2 cells under normoxic or hypoxic conditions. AD, over-expression of Hsp90 β using adenovirus; shRNA, Hsp90 β knockdown using shRNA plasmids; H12, hypoxia (1% O₂) for 12 hrs. *P < 0.05, versus normoxic controls; #P < 0.05, versus hypoxia control group; n = 3.

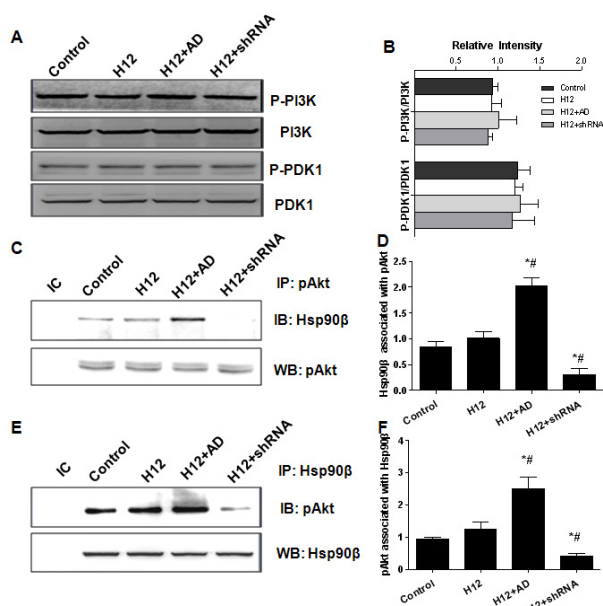


Fig. 4. Hsp90 β promotes Akt phosphorylation not through PI3K/PDK1/Akt pathway but through stabilizing pAkt. (A, B) Examples and quantitation of Western blots of PI3K, p-PI3K, PDK1 and p-PDK1 showing no change of the abundance or p-PI3K and p-PDK1 by hypoxia or by altering Hsp90 β expression. (C, D) Examples and the quantitation of Hsp90 β associated with pAkt. Total cell lysate was precipitated with an antibody against pAkt followed by immunoblot analysis with anti-pAkt and anti-Hsp90 β . (E, F) Examples and the quantitation of pAkt associated with Hsp90 β in cells with different expression levels of Hsp90 β . AD, over-expression of Hsp90 β using adenovirus; shRNA, Hsp90 β knockdown using shRNA plasmids; H12, hypoxia (1% O₂) for 12 hrs. *P < 0.05, versus controls; #P < 0.05, versus hypoxia control group; n = 3. IP immunoprecipitation, IB immunoblotting, IC isotype control.

DISCUSSION

Under physiological conditions, IEC apoptosis balancing IEC proliferation is the major mechanism responsible for intestinal epithelium turnover (3). However, excess IEC apoptosis could be pathogenic in that the loss of IECs as a result of shedding of apoptotic cells may compromise epithelial integrity and increase host susceptibility to infection (23). IEC apoptosis can be induced by gastrointestinal hypoxia associated with trauma, shock or severe burns and under these conditions gastrointestinal sepsis develops (24). In this study, we sought to determine the role of Hsp90 β in IEC apoptosis induced by hypoxia and the underlying mechanism using the IEC cell line Caco2. Our results show that hypoxia (1% O₂) resulted in the apoptosis of the Caco2 cells in a time-dependent manner. Hsp90 β provides the protective effect on IECs by enhancing pBAD, which reduces cytochrome C release, subsequent caspase 3 activation and PARP cleavage. This protective effect of Hsp90 β is through directly interacting with pAkt and stabilizes it.

Hsp90 β exerts a protective effect on IEC apoptosis induced by hypoxia

Hsp90 has a protective effect against apoptosis in many types of cells (12) but does not provide protection in other types (25). For IECs, it is still unknown if Hsp90 β has protective effect in intestinal epithelium injury or IEC apoptosis. Our study clearly showed that Hsp90 β is able to inhibit hypoxia-induced apoptosis of enterocyte-like Caco2 cells. Hsp90 has α and β isoforms encoded by two genes but their functional difference is poorly defined. Our study suggested that Hsp90 β could play an important role in anti-apoptosis of Caco2 cells induced by hypoxia. We used cultured IEC-like cells to test our idea *in vitro*. It warrants further study to explore the role of Hsp90 β in the intestine under ischemia/reperfusion condition *in vivo*.

The role of Akt in cytoprotection offered by Hsp90 β

The Akt signaling pathway is now recognized as one of the most critical pathways in regulating cell survival to withstand apoptotic stimuli (26). In our study, the level of pAkt is increased in hypoxic Caco2 cells, probably as a counteraction against the detrimental effects of hypoxia, although it could not completely inhibit Caco2 cell apoptosis induced by hypoxia. The beneficial effect of pAkt against Caco2 cell apoptosis induced by hypoxia was revealed by the fact that inhibition Akt with LY294002 or reducing pAkt by knockdown Hsp90 β greatly increased apoptosis of Caco2 cells under hypoxia. When pAkt was further increased by overexpressing Hsp90 β , it offered substantial protection to hypoxic Caco2 cells. Therefore, our findings suggest that Akt plays a major role in the protection of hypoxia-stressed IECs.

The mechanism of increasing pAkt by Hsp90 β

Activated PI3K catalyzes the production of PtdIns-(3,4)-P2 and PtdIns-(3,4,5)-P3 helping to recruit Akt and PDK1 to the membrane, where it is phosphorylated by PDK1 (12). In this study, we found that both the expression and phosphorylation of PI3K and PDK1 were not changed by Hsp90 β and thus the mechanism of increased pAkt by Hsp90 β overexpression is independent of PI3K/PDK1. Our study implied that in Caco2 cells under hypoxic conditions, Hsp90 β increases pAkt by stabilizing it, supporting the findings in previous reports in endothelial cells (14). An apparent issue is how this occurs when total Hsp90 β has not been significantly increased by hypoxia. One possibility is that hypoxia induces the translocation of Hsp90 to microtubules to form a complex with pAkt and stabilize it as previous studies suggests. Hsp90 does not stabilize pAkt unless it is recruited by microtubules (27).

In conclusion, our data collectively indicate that hypoxia induces apoptosis of the Caco2 cells in a time-dependent manner, during which Hsp90 β plays an important role in protecting the Caco2 cells from the hypoxia-induced injury. This is achieved by stabilizing pAkt that forms a complex with Hsp90 β . Activated Akt then phosphorylates BAD to prevent it from activating the apoptotic signaling pathway.

MATERIALS AND METHODS

Cell culture and hypoxia treatment

Caco2 cells, an intestinal epithelial cell line, were grown in DMEM supplemented with 10% (V/V) heat-inactivated fetal bovine serum (FBS), penicillin G (100 U/ml) + streptomycin (100 mg/ml) before hypoxia treatment. Then a PKA inhibitor (V5681, 10 μ M), an Akt inhibitor (LY294002, 30 μ M), a PKC inhibitor (V5691, 10 μ M) and a MAPK inhibitor (U0126, 10 μ M), or an equal volume of vehicle (dimethyl sulfoxide) were added to the Caco2 cells and incubated at 37°C for 1 hour before hypoxia. These drugs were purchased from Promega.

Hypoxia treatment was performed as described previously (5). Briefly, immediately before exposure to hypoxia, the cell culture medium was changed to serum-free DMEM and the hypoxic condition was achieved by using a hypoxic incubator (Thermo, USA) which was filled with a gas mixture of 94% N₂, 5% CO₂, and 1% O₂. Cells were subjected to hypoxic conditions by replacing the normoxic medium with the hypoxic medium and continued to culture.

Recombinant Hsp90 β adenovirus (AdHsp90 β) and shRNA plasmid construction and transduction

The Hsp90 β plasmid was gifted by Dr. Workman (Institute of Cancer Research, 237 Fulham Road, London, UK). The recombinant adenoviruses were prepared using the AdEasyTMxL system (Stratagene, La Jolla, USA) according to the manufacturer's instruction. The Caco2 cells were initially cultured in DMEM with 10% FBS. Then the medium was changed to a medium without FBS before being infected with adenoviruses at a multiplicity of infection (MOI) of 10-20 particles/cell for 24 h. The cells were then cultured in DMEM with 10% FBS before morphological or biochemical analysis. The expression of Hsp90 β mediated by AdHsp90 β in the Caco2 cells was confirmed by Western blotting.

This study used two Hsp90 β shRNAs targeting two special regions of the Hsp90 β transcript, and two scrambled control shRNAs. The sequences of Hsp90 shRNAs were:

• 5'TGCTGTAAGTACTCTGTCTGGTCTTCGTTTTGGCCACTGACTGACGAAGACCACAGAGTACTTA3'

• 5'CCTGTAAGTACTCTGTGGTCTTCGTCAGTCAGTGGCCAAACGAAGACCAGACAGAGTA CTTAC3'.

And the sequences of the control shRNAs were:

• 5'TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT3'

• 5'CCTGAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAACCGTCTCCACGCAGTACATTT3'.

These shRNA fragments were ligated into a pcDNATM6. 2-GW/EmGFP plasmid (Invitrogen) individually. The Caco2 cells were seeded at a density of 3. 0 \times 10⁴ cells per well in 24-well plates. The stable Caco2 clones were selected with medium containing Blasticidin S HCl (5 μ g/ml, Invitrogen) for 3 weeks and the expression levels of Hsp90 β protein were determined by Western blot.

Flow Cytometric Analysis of Apoptosis and Caspase 3 activity measurement

Early stage cell apoptosis was evaluated by staining cells with the AnnexinV-APC/PI Apoptosis Detection Kit (KeyGEN Biotech, China) according to the manufacturer's instructions (AnnexinV-APC/PI positive). The cells floating in the culture supernatants were collected by centrifugation and pooled with adherent cells recovered from the plates. Caspase 3 activity was measured using a colorimetric assay kit (KeyGEN Biotech, China).

Terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick end-labelling (TUNEL) assay

DNA strand breaks in apoptotic cells can be revealed by TUNEL staining. TUNEL was performed on the Caco2 cells using an In Situ Cell Death Detection Kit (Roche Molecular Biochemicals, IN). Total nuclear number and TUNEL positive nuclear number were counted from at least 1,000 cells in each group for each culture at \times 20 with a microscope (28).

Western blot analysis

Cells were harvested, washed for 3 times with ice-cold phosphate-buffered saline (PBS) and lysed in an SDS sample buffer at 4°C. Whole-cell lysates were prepared and total protein concentrations were measured using an RDC Protein Assay. Proteins were analyzed with Western blot using primary antibodies against Hsp90 β (Abcam), PI3K, p-PI3K, PDK1, p-PDK1, Akt, pAkt, cytochrome C, BAD, pBAD and cleaved PARP (all from Cell Signaling Technology), cleaved caspase 3 (Santa Cruz) and GAPDH (Sigma-Aldrich). Horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG (Santa Cruz) was used as the secondary antibody. GAPDH was used as a loading control. Immunocomplexes were visualized using enhanced chemiluminescence (GE, USA) according to the manufacturer's instructions.

Co-immunoprecipitation

Cells were washed for 3 times with PBS and lysed for 30 min in 500 μ l RIPA buffer with 2 mM PMSF and a protease inhibitor cocktail at 4°C. Samples were precleaned for 1 h at 4°C using protein G-Sepharose (GE). Then 30 μ g total proteins were incubated with pAkt antibody (Cell Signaling Technology) or Hsp90 β antibody (Abcam) and protein G-Sepharose overnight at 4°C. The G-Sepharose beads were centrifuged down and then washed for three times with RIPA buffer. Proteins were released by boiling for 5 min in SDS sample buffer and fractionated by SDS-PAGE. Then, blots were probed with the intended antibody. Experiments were performed for at least three independent times.

Statistical analysis

Data were presented as the mean \pm SEM. SPSS 13. 0 was used for statistical analysis with one-way or two-way ANOVA followed by Tukey Multiple Comparison tests. P values \leq 0.05 were considered statistically significant.

Acknowledgements

This study was supported by the State Key Lab Funding in China (SKLZZ200905), CPLA Scientific Research Fund (BWS11J039) and Key Project on Advanced Clinical Technology for the Military Hospital (2010gxjs068).

REFERENCES

1. Taylor, C. T. and Colgan S. P. (2007) Hypoxia and gastrointestinal disease. *J. Mol. Med.* **85**,1295-1300.
2. Han, X., Ren X., Jurickova, I., Groschwitz, K., Pasternak, B. A., Xu, H., Wilson, T. A., Hogan, S. P. and Denson. L. A. (2009) Regulation of intestinal barrier function by signal transducer and activator of transcription 5b. *Gut* **58**, 49-58.
3. Edelblum, K. L., Yan, F., Yamaoka, T. and Polk, D. B. (2006) Regulation of apoptosis during homeostasis and disease in the intestinal epithelium. *Inflamm. Bowel. Dis.* **12**, 413-424.
4. Van Leeuwen, P. A., Boermeester, M. A., Houdijk, A. P., Ferwerda, C. C., Cuesta, M. A., Meyer, S. and Westorp, R. I. (1994) Clinical significance of translocation. *Gut* **35**, 28-34.
5. Wang, W., Peng, Y., Wang, Y., Zhao, X. and Yuan, Z. (2009) The anti-apoptotic effect of heat shock protein 90 on hypoxia-mediated cardiomyocyte damage through the PI3K/Akt pathway. *Clin. Exp. Pharmacol. Physiol.* **36**, 899-903.
6. Friedman, G. B., Taylor, C. T., Parkos, C. A. and Colgan, S. P. (1998) Epithelial permeability induced by neutrophil transmigration is potentiated by hypoxia: role of intracellular cAMP. *J. Cell. Physiol.* **176**, 76-84.
7. Ikeda, H., Suzuki, Y., Suzuki, M., Koike, M., Tamura, J., Tong, J., Nomura, M. and Itoh, G. (1998) Apoptosis is a major mode of cell death caused by ischaemia and ischaemia/reperfusion injury to the rat intestinal epithelium. *Gut* **42**, 530-537.
8. Sreedhar, A. S., Kalmar, E., Csemely, P. and Shen, Y. F. (2004) Hsp90 isoforms: functions, expression and clinical importance. *FEBS Lett.* **562**, 11-15.
9. Prodromou, C., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W. and Pearl, L. H. (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90**, 65-75.
10. Musch, M. W., Ciancio, M. J., Sarge, K. and Chang, E. B. (1996) Induction of heat shock protein 70 protects intestinal epithelial IEC-18 cells from oxidant and thermal injury. *Am. J. Physiol.* **270**, 429-436.
11. Holzbeierlein, J. M., Windsperger, A. and Vielhauer, G. (2010) Hsp90: a drug target? *Curr. Oncol. Rep.* **12**, 95-101.
12. Fujita, N., Sato, S., Ishida, A. and Tsuruo, T. (2002) Involvement of Hsp90 in signaling and stability of 3-phosphoinositide-dependent kinase-1. *J. Biol. Chem.* **277**,10346-10353.
13. Vivanco, I. and Sawyers, C. L. (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat. Rev. Cancer* **2**, 489-501.
14. Zhang, R., Luo, D., Miao, R., Bai, L., Ge, Q., Sessa, W. C. and Min, W. (2005) Hsp90-Akt phosphorylates ASK1 and inhibits ASK1-mediated apoptosis. *Oncogene* **24**,3954-3963.
15. Gray, P. J. Jr., Stevenson, M. A. and Calderwood, S. K. (2007) Targeting Cdc37 Inhibits Multiple Signaling Pathways and Induces Growth Arrest in Prostate Cancer Cells. *Cancer Res.* **67**,11942-11950.
16. Basso, A. D., Solit, D. B., Chiosis, G., Giri, B., Tschlis, P. and Rosen, N. (2002) Akt Forms an Intracellular Complex with Heat Shock Protein 90 (Hsp90) and Cdc37 and Is Destabilized by Inhibitors of Hsp90 Function. *J. Biol. Chem.* **277**, 39858-39866.
17. Arsham, A. M., Plas, D. R., Thompson, C. B. and Simon, M. C. (2002) Phosphatidylinositol 3-kinase/Akt signaling is neither required for hypoxic stabilization of HIF-1 alpha nor sufficient for HIF-1-dependent target gene transcription. *J. Biol. Chem.* **277**, 15162-15170.
18. Sakamaki, J., Daitoku, H., Ueno, K., Hagiwara, A., Yamagata, K. and Fukamizu, A. (2011) Arginine methylation of BCL-2 antagonist of cell death (BAD) counteracts its phosphorylation and inactivation by Akt. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 6085-6090.
19. Koh, P. O. (2011) Nicotinamide attenuates the ischemic brain injury-induced decrease of Akt activation and Bad phosphorylation. *Neurosci. Lett.* **498**,105-109.
20. Zhang, L., Zambon, A. C., Vranizan, K., Pothula, K., Conklin, B. R. and Insel, P. A. (2008) Gene expression signatures of cAMP/protein kinase A (PKA)-promoted, mitochondrial-dependent apoptosis. Comparative analysis of wild-type and cAMP-deathless S49 lymphoma cells. *J. Biol. Chem.* **283**, 4304-4313.
21. Chong, Z. Z., Kang, J. Q. and Maiese, K. (2002) Erythropoietin is a novel vascular protectant through activation of Akt1 and mitochondrial modulation of cysteine proteases. *Circulation* **106**, 2973-2979.
22. Sato, S., Fujita, N. and Tsuruo, T. (2000) Modulation of Akt kinase activity by binding to Hsp90. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10832-10837.
23. Abreu, M. T., Palladino, A. A., Arnold, E. T., Kwon, R. S. and McRoberts, J. A. (2000) Modulation of barrier function during Fas-mediated apoptosis in human intestinal epithelial cells. *Gastroenterology* **119**,1524-1536.
24. Hotchkiss, R. S., Tinsley, K. W. and Karl, I. E. (2003) Role of apoptotic cell death in sepsis. *Scand. Infect. Dis.* **35**, 585-592.
25. Galea-Lauri, J., Richardson, A. J., Latchman, D. S. and Katz, D. R. (1996) Increased heat shock protein 90 (hsp90) expression leads to increased apoptosis in the monoblastoid cell line U937 following induction with TNF-alpha and cycloheximide: a possible role in immunopathology. *J. Immunol.* **157**, 4109-4118.
26. Beere, H. M. (2004) The stress of ding': the role of heat shock proteins in the regulation of apoptosis. *J. Cell Sci.* **117**, 2641-2651.
27. Giustiniani, J., Daire, V., Cantaloube, I., Durand, G., Pous, C., Perdiz, D. and Baillet, A. (2009) Tubulin acetylation favors Hsp90 recruitment to microtubules and stimulates the signaling function of the Hsp90 clients Akt/PKB and p53. *Cell Signal* **21**, 529-539.
28. Zhang, K. R., Liu, H. T., Zhang, H. F., Zhang, Q. J., Li, Q. X., Yu, Q. J., Guo, W. Y., Wang, H. C. and Gao, F. (2007) Long-term aerobic exercise protects the heart against ischemia/reperfusion injury via PI3 kinase-dependent and Akt-mediated mechanism. *Apoptosis* **12**,1579-1588.